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Phononics and Micromechanics of Bio-Colloidal Wiseana Iridovirus

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***Abstract*-By using Brillouin Light Scattering (BLS), we have investigated phononic properties of Wiseana iridovirus (WIV) assemblies and dispersed individual viruses at hypersonic frequency window. Propagating modes in virus assemblies and localized vibrational eigenmodes of individual virus have been identified. Based on phonon spectra, Young's modulus of the virus has been estimated to be ~ 7 GPa, suggesting that the WIV virions are mechanically more similar to their DNA cores than to their capsid (protein) shells. The results also indicate that viral colloids are mechanically coupled during assembly in contrast to a system of synthetic polymeric colloids.**

BACKGROUND

Viruses, the most abundant biological entities on the planet, are attractive for fabrication of the next generation of nano-electronic and optoelectronic devices due to their extreme mono-dispersity, geometrical symmetry, and specific chemistry [1, 2]. Knowledge of mechanical properties of viruses is recognized to be important not only to understanding the relationship between structure and bio-function of viruses but also to development of more sophisticated nano-devices. Virus capsids are expected to be robust enough to withstand high internal pressure from dense nucleic acid packing inside which would facilitate the genome injection into the prospective host cell in the viral infection cycle, at the mean time, must be flexible enough to release them. Recently, the mechanics of virus capsids have been investigated experimentally and theoretically [3, 4]. However, diversity of the experimental tools is still limited largely due to their small sizes and complex structures. Brillouin (inelastic) light scattering as a direct, non-contact and non-destructive measurement of phononic and mechanic properties has been successfully applied to photonic, phononic crystals and polymeric nano-structures recently [5, 6]. Here, we report BLS studies of phonon behaviors and mechanical properties of individual Wiseana Iridovirus and its assembly in comparison with polymeric colloids.

MATERIALS AND METHODS

The WIV has an icosahedral symmetry with flat sides about 140 nm apart. There are fibrils about 30 nm long protruding from the shell of virus. A lipid bilayer of about 4 nm thickness is underneath the capsid shell, enclosing the DNA core. The preparation and assembly of WIV virion can be found in the previous literature [7]. Virus assembly has been fabricated on silicon with a thickness of about 1 μm. The viruses were stored and measured at temperatures between 19-24 °C and relative humidities between 10-30%.

Polymethyl methacrylate (PMMA) colloids of diameter about 300 nm were deposited onto an aluminum mirror.

BLS spectra of virus on silicon were measured in backscattering geometry with a tandem Fabry-Perot interferometer (Sandercock model) and an argon-ion laser (Lexel 3500 with a frequency lock mode). The wavelength λ is 514.5 nm and power is in the range of 10-30 mW. The free spectral ranges of the interferometer were taken at 30, 50 and 75 GHz to obtain high resolution and/or broad frequency range. The scattering vector along the surface $Q_{||} = (4\pi/\lambda)\sin\theta$ was varied by change of the incident angle θ with respect to the surface normal (θ = 15º-75º). The polarization of the incident beam was chosen to be perpendicular to the scattering plane (vertical). The polarization of scattered light was either vertical (polarized scattering) or no analyzer was used. The Brillouin peaks were fit with Lorentzian function.

RESULTS

PMMA colloid crystal as shown in SEM picture (Figure 1a) was investigated for comparison because these two colloidal systems (PMMA hard spheres and virus particles) may have some similarities in phononic properties. The BLS spectra show several distinct peaks (Figure 1b), which are Q-independent. These non-propagating localized modes are identified as vibration eigenmodes of PMMA hard spheres. Based on the lowest frequency mode, the transverse sound velocity (V_t) of PMMA hard sphere with a diameter D (300 nm) can be calculated according to following expression, which is 1.40 km/s, consistent with 1.42 km/s of bulk PMMA.

$$\nu_{ln} \approx 0.85\frac{V_t}{D}$$

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The virus assembly shows blue iridescence, which origins from light reflected from this bio-colloidal photonic crystal. In the Brillouin spectra of thick virus assembly (Figure 2a), it was found that the modes were $Q_{\parallel}$-dependent in contrast to those from PMMA colloid crystal. This $Q_{\parallel}$-dependence clearly suggests that there exist surface waves propagating in the virus assembly which could arise from the interlocks of fibril on the surface of virus. This may also lead to the difficulty in fabricating uniform, defect-free viral assemblies as previously reported. The dispersion relationship of the modes is illustrated in Figure 2d. The modes with lowest sound velocity can be identified as Rayleigh modes which approach the asymptotic Rayleigh velocity of 1.44 ± 0.10 km/s at high scattering wavevector $Q_{\parallel}$ based on calculation from classical wave theory for a soft film on a hard substrate and assumption of Possion's ratio of 0.33 which is reasonable for biological objects [8].

The BLS spectra of individual viruses are shown in Figure 2b. Besides the Rayleigh mode of the silicon substrate, another weak, $Q_{\parallel}$-independent localize modes at 8.8 ± 0.2 GHz can be observed, which dispersion relationship is shown in Figure 2d. This is significantly different from that of the thick viruses assembly, while is similar to the localized mode of PMMA colloid except lower intensity of the damped peak. The transverse sound velocity is calculated to be 1.45 ± 0.05 km/s by assuming a sphere of the virus particle with a diameter of 140 nm which is consistent with that in thick virus assemblies.

Young's modulus can be obtained from sound velocities:

$$E = \rho V_t^2 \left[\frac{3V_l^2 - 4V_t^2}{V_l^2 - V_t^2} \right]$$

V_l is longitudinal sound velocity, ρ is effective density of the virus. By simplification of the structure of the virus with ~ 4 nm thick outer layer having the same density 1.21 as protein lysozyme and DNA core of a radius ~ 66 nm, the volume fraction of DNA core is estimated to be 83%. ρ can be approximated according to the linear addition law. The density of DNA is reported to be in a range of 1.21 ~ 1.6, which results in an estimation of Young's modulus 6.7 ~ 8.9 GPa.

Our estimate of WIV modulus is a few times larger than the value of other virions estimated from AFM measurements [4]. This difference might originate from structural differences between WIV and other virions. Another possible reason is thought to be the distinct differences in the measurement technique and conditions. In BLS experiment, high frequency (GHz) or instantaneous modulus is measured, while AFM measurements were preformed at much lower frequencies. This difference is usually attributed to relaxation processes. In our experiment, virions in humid environment were characterized while the AFM measurements were performed in solutions. We emphasize that Brillouin technique as a non-contact and non-destructive method is promising to have a broad application in biological materials and nanomaterials.

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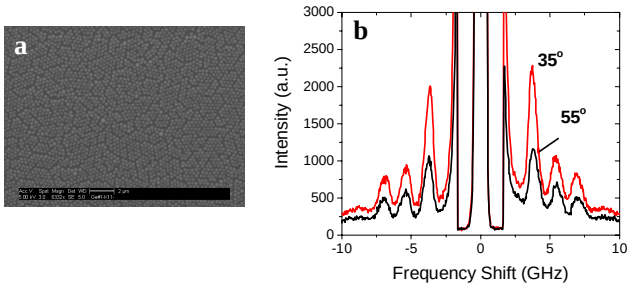

Fig. 1. (a) SEM of a monolayer of PMMA colloids (D = 300 nm) on an aluminum mirror. (b) Brillouin spectra of PMMA colloids at scattering angle of 35° and 55°.

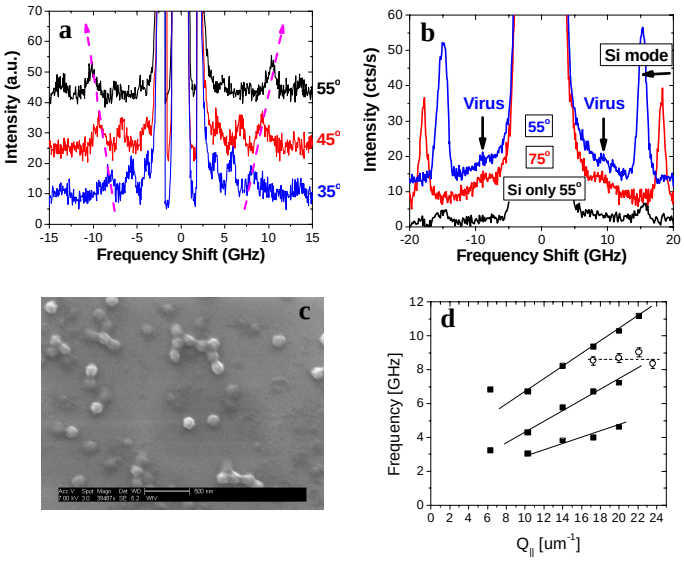

Fig 2. (a) Brillouin spectra of the thick virus assemblies at scattering angles 35°, 45°, 55°. (b) Brillouin spectra of individual viruses on Si at scattering angle of 55° and 75°. Eigenmode of individual viruses is marked. (c) SEM of the dispersed viruses on Si. (d) Dispersion plots of frequency *versus* $Q_{\parallel}$. The solid squares are for thick virus assembly; the empty circles are for the single virus measurements. The solid lines are guides for the eyes.